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Hypothesis

Specificity out of clutter: A hypothetical role of G protein-coupled receptors in the non-genomic effect of steroids

Chen Wang [a], Yi-Jia Li [a], Ji-Min Cao [b,*]

[a] Department of Medicine, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, China
[b] Department of Physiology and Pathophysiology, Institute of Basic Medical Sciences Chinese Academy of Medical Sciences, School of Basic Medicine Peking Union Medical College, Beijing, China

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ABSTRACT

The non-genomic effect has been considered to underlie the rapid action of steroids. This signaling is initiated at the plasma membrane-level and does not directly influence gene expression. Recent studies have provided detailed information on their downstream pathways, but less is known about the nature of correlated membrane-bound receptors. Here, we propose that binding of steroids to a consensus motif, namely CRAC, of G protein-coupled receptors (GPCRs) shifts the agonist-binding state of receptors and accounts for this effect to a certain extent. The interaction between steroids and GPCRs is specific, while the identities of the GPCRs involved are not restrained, which can coordinate the high heterogeneity of this signaling and reconcile multiple discrepancies in the literature.

1. Introduction

Steroid hormones (hereafter abbreviated as steroids), essential components of the endocrine system, modulate many physiological and pathological processes. They have long been recognized to exert both rapid and delayed effects. The delayed effect, according to the classical model of steroids action, is mediated by cytosolic steroid receptors, which takes a time lag of hours or even days. These ligand–receptor complexes translocate into the nucleus and regulate gene expression by binding to response elements in promoter regions. Steroids can also exert some rapid, transcription-independent actions in seconds or minutes after their application. These so called 'non-genomic effects' have been noted for at least a half-century [2]. Non-genomic effects do not influence gene expression, but drive more rapid biological actions via signal transduction. Examples of these non-genomic effects include estrogen mediated blood vessel dilation, progesterone induced acrosomal action and glucocorticoid triggered tracheal relaxation [3].

There is growing body of evidence to suggest that these acute actions are mediated by specific receptors localized most often to the plasma membrane. Membrane impermeable steroid conjugates, such as BSA–steroid and polymer–steroid, can still perform these rapid actions, which seem to be the most direct support for the membrane-initiated steroid signaling (MISS). Although well-defined in several biological models, the nature of these receptors is still elusive, triggering extensive research. The search for membrane receptors has been ongoing for many years, and several candidates have been isolated via radio-labeled ligand–receptor purification from membrane fractions [4]. However, none of them has been unequivocally confirmed as the functional receptor. These attempts were hampered by the lipophilic nature of steroids, resulting in highly nonspecific binding [5]. Hitherto, several types of candidates have been proposed for the non-genomic effects, including classical steroid receptors localized in the plasma membrane, traditional G protein-coupled receptors (GPCRs) and novel membrane-associated steroid-binding proteins [6]. Approximately 5–10% endogenous estrogen receptor α (ERα) are present at the plasma membrane that has been suggested to mediate the rapid signaling of estrogen [7]. Compared to membrane localized classical steroid receptors, GPCRs are more appealing candidates (Table 1). Pertussis toxin, which locks Gi protein in the GDP-bound form, inhibits the non-genomic action of steroids in several models which implies the involvement of G protein-GPCR signaling [8]. The GPCR hypothesis reached a peak when GPR30 was identified as the membrane bound estrogen receptor with high affinity, although further evidence indicated its localization at the endoplasmic reticulum [9]. Furthermore, a GPCR-like protein, namely membrane progestin receptor (mPR) has been isolated and characterized by using one of the most relevant non-genomic effect models, steroid-induced oocyte maturation [10]. To reconcile the existing data, perhaps both classical steroid receptors and newly

* Corresponding author. Fax: +86 10 6915 6467.
E-mail address: caojimin@126.com (J.-M. Cao).

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Table 1
Steroid hormones, non-genomic effects and specific GPCRs possibly involved.

Steroid	Non-genomic effect	Specific GPCR
Estrogen	Vasodilitation Breast cancer, endometrial cancer and ovarian cancer cell proliferation and survival	GPR30 [9]
Progesterone	Fish oocyte maturation	mPR [10]
Androgen	Prostate cancer cell proliferation and survival Frog oocyte maturation	GPRC6A [11]

described GPCRs are responsible for non-genomic effects, depending on the biological processes and involved steroids. But the question of how these highly hydrophobic steroids specifically interact with GPCRs that contain no known steroid-binding domain has not been answered.

To address this concern, we provide a novel and provocative insight toward the interaction between steroids and GPCRs, in trying to explain non-genomic effects of steroids.

2. The hypothesis and evaluation

Although no conserved steroid binding pocket has been recognized in GPCRs, a consensus binding motif was defined for cholesterol, which shares the mother nucleus with steroids. This motif, named cholesterol recognition/interaction amino acid consensus sequence (CRAC), was first recognized in the peripheral-type benzodiazepine receptor [12]. In addition to biochemical characterization, the existence of this binding motif was further confirmed when the crystal structure of human β2 adrenergic receptor-cholesterol was resolved [13]. In this structure, cholesterol did not dock to the ligand-binding pocket of GPCR, but bound in a shallow groove formed by segments of helices I, II, III and IV, which in the view of structural biology enhanced the thermal stability. The interaction was defined by four spatially distributed amino acids, and further alignment analysis revealed that CRAC was conserved in about one-third of class A GPCRs. This implies that cholesterol binding influences the properties of GPCRs. Indeed, modulatory effect of cholesterol has been demonstrated for the oxytocin receptor, in which cholesterol not only enhanced its thermal stability but shifted the receptor to a high-affinity binding state. Moreover, a variety of sterol analogues have also been shown to regulate the agonist-binding affinity of the oxytocin receptor [14]. Steroids, derived from cholesterol with partial cleavage of the carbon-17 side chain, are considered to be more flexible ligands for the binding site. In the light of these data, we propose that the non-genomic effect of steroids is mediated by the interaction between steroids and GPCRs via CRAC, which further influences the receptor activity. The interaction between steroids and CRAC is specific, while the GPCRs involved need not be specific, i.e. dozens of GPCRs bearing CRAC will be regulated by the steroids. This pan-interaction can accommodate the unexplained controversies in literatures: (i) different steroids can exert a similar non-genomic effect, as both progestin and androgen can promote oocyte maturation [6], (ii) for a single steroid, various downstream signaling pathways have been implicated, including adenylate cyclase-protein kinase A (PKA) or phospholipase C (PLC)-protein kinase C (PKC) [3], (iii) different effects are initiated by same steroid in various models, which can be explained by the compositional difference of GPCRs' pool expressed in distinct cell types, and (iv) there is no strict enantioselectivity for the steroid effect [15] in accordance with the observed promiscuity of cholesterol-binding site for a series of sterol analogues [14]. This hypothesis can be regarded as a general mechanism to explain the highly variable non-genomic effects and also the multiple failures in the search for specific membrane receptors for most steroids.

Given the lipophilic nature of steroids, their influence on membrane fluidity and curvature may also contribute to the non-genomic effect via GPCRs. As seven transmembrane proteins, GPCRs' activity is also regulated by the microenvironment formed by lipid bilayers. It has been well-established that cholesterol–rhodopsin interaction can promote a photon receptor shift between different intermediate states [16]. Steroids can also imitate this effect.

3. Conclusions

The non-genomic effect of steroids is highlighted as a membrane-associated signaling, which makes it distinct from the classical steroid action on gene expression. We propose that the specific interaction between steroids and CRAC of unconstrained GPCRs accounts for this effect to some extent. This can also be attributed to their influence on biophysical properties of the plasma membrane. We hope this hypothesis can provide a novel insight for the allosteric regulation of steroids signaling.

Disclosures

None.

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